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Studies on the Parasitic Nematode Protospirura Muris

STUDIES ON THE PARASITIC NEMATODE PROTOSPIRURA MURIS (GMELIN 1790)

BY

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Duncan C. Hetherington

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BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF Master of Arts

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In Charge of Thesis

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Foreword

This piece of work was undertaken in the Laboratory of Parasitology at the University of Illinois, under the direction of Doctor Henry B. Ward, to whom the writer wishes at this time to express his sincere appreciation of the interest shown in the carrying out of this study.

STUDIES ON THE PARASITIC NEMATODE PROTOSPIRURA MURIS (GMELIN 1790)

I Introduction

(a) History and Classification

The nematode as the subject of study in this paper was first classified by Gmelin in 1790 in the book written by him shortly after the death of his teacher Linnaeus. In this "Caroli a Linné systema naturae," it is the twentieth species of Ascaris; namely, Ascaris muris accompanied by the following brief description: "Cauda pennata, intestino obscuriori. Habitat in musculi ventriculo, annulata, feminae genitalibus triplici foramine biantibus." (Tail winged, intestine obscured. Habitat in the stomach of the mouse. Body annulated, and the female genitalia of three parts opening midway between the ends of the body.) He also made reference to books of Werner and Goeze. I was unable to see the former author's book but evidently he makes mention of a worm perhaps the same one as that or a similar one to that of Gmelin, naming it Lumbricus muris. Goeze on the other hand treats of a nematode found in the stomach of a certain falcon and other birds, calling it Ascaris teretes. At the same time he mentions having received a letter from Graf von Borke telling of his finding four Ascaris teretes from the stomach of a male mouse and five of the same from the intestine of the large-eared bat. Obviously they cannot be the worm and it is most unlikely that either of the groups could be the same as Ascaris teretes since the latter as Goeze states were found in

birds. Also there is no descriptive proof that the nematodes found in the mouse by Graf von Borke were the same as <u>Ascaris muris</u> of Gmelin. Hence by right of priority the specific name of the nematode of this paper is <u>muris</u>, attributed to Gmelin.

Later authors gave the worm various other classifications. According to Hall, Fröelich in 1791 called the same nematode from the stomach of the mouse, <u>Ascaris obtusa</u>, while Zeder in 1803 named it <u>Fusaria muris</u> (Gmelin 1790), which name is mentioned by Rudolphi (1809) in connection with his description of <u>Ascaris obtusa</u> (Fröelich). According to Marchi and Hall, Rudolphi in 1809 classified this nematode as <u>Spiroptera obtusa</u> (Fröelich). Then in 1866 Schneider changed that generic name to <u>Filaria</u> while Stössich (1897) renamed it specifically as <u>Filaria muris</u> (Gmelin). In the same year von Linstow called the self-same nematode collected in Madagascar, <u>Spiroptera Brauni.</u>

Finally Seurat in 1914, after studying the new nematode from the stomach of <u>Felis ocreata</u>, noticed in it a more primitive organization than would admit it to the genus <u>Spiroptera</u>. Furthermore it was not closely enough related to lower groups, so in consequence he created for it the new genus <u>Protospirura.</u> Later after further study of the group <u>Spiroptera</u>, particularly embryologically, he included in the new genus the nematode <u>Spiroptera obtusa Rud.</u>, previously mentioned, calling it <u>Protospirura muris</u> (Gmelin 1790).

(b) Hosts and Distribution

Ordinarily this nematode has been described as inhabiting the stomach of its host, the common mouse, <u>Mus musculus.</u> It has however been reported from the same organ in the brown rat--<u>Mus decumanus</u>, the Alexandrian rat--<u>Mus alexandrinus</u>, <u>Mus sipronus</u>, the common rat--<u>Mus rattus</u>, and the field mouse--<u>Apodemus sylvaticus.</u>

As for geographical distribution, this particular parasite has been collected from hosts in Africa, Brazil, Australia, Continental Europe, and North America.

II Methods of Investigation

(a) Collection and Preservation of Material

The material for study in this paper was collected from the common house mouse, Mus musculus. The hosts were obtained from houses in Urbana and Champaign, Illinois, but principally from the horse stables and dairy barns of the University of Illinois. Mice were caught during the fall months from October 15 to November 24, 1919; once in January and twice in February; then, again, continuously thruout April, 1920. In all, 81 specimens of mice were examined, 80 of which were the common mouse and one of the genus Microtus. At the same time in another piece of work being carried on in the laboratory 42 specimens of Mus rattus were examined with note of only one infection of a single specimen of Protospirura murus. In the case of the mice examined infections were noted in eighteen cases or 22.2% of the total of 81 hosts. It is interesting to note also that in the mice caught and examined in the fall all the specimens of the nematode with one or two exceptions were mature or nearly so, while in the spring the infections, excepting one of three mature female worms, were of very recent origin since they yielded often nematodes only a few millimeters long; also of the total of 93 nematodes collected, 47 of those or over 50% came from 3 hosts which yielded 13, 19, and 15 specimens respectively--the other animals yielding from one to seven nematodes each. Thus it is seen that in general this nematode is not at all plentiful. Female worms were always more abundant than the males and many times an infection consisted only of female worms. With such an occurrence it is readily seen that chances for reproduction of its kind are very few and far between, thus, in part, accounting for the relative scarcity of the worm.

In every case of infection the worms were found in the stomach of the host, and where only small numbers were found the stomach did not differ appreciably in appearance from that of a normal mouse. But with the three cases of heavy infection mentioned above, the stomach was greatly enlarged, very thin walled, and permanently distended by the worms which were clearly visible thru the delicate tissues. Besides the distension the organ presented no lesions which were noticeable.

For observation of the nematodes in the living state, it was found that they seemed to keep best in distilled water just acidulated with hydrochloric acid. On one occasion four female worms were removed early in the morning from the stomach of a frozen mouse and were apparently dead. They were placed in acidulated water and after a few moments showed signs of life. After the water was slightly warmed they became quite lively and when later transferred to some lactose nutrient broth they remained active thruout the day. In the evening they were transferred to a medium of starch agar and lactose broth in a petri dish and incubated presumably at 37° C. They were active the following day at noon but later died. As the experiment was performed without any premeditation, no means were available at the time for making the cultures sterile and regulating the incubator, which unavoidably ran up to 40° C. Hence with a heavy bacterial contamination and this excessive heat, it is little wonder the nematodes succumbed. However, the experiment was merely mentioned here as hinting at a possible means of artificial culture for the parasite. Except for the observation of movements on the part of the nematodes, there is little gained by the study of the living specimens since, with the exception of the caudal and cephalic regions, they are too

opaque for observing internal organization and structure.

For killing and fixing the nematodes various mixtures were experimented with, such as Kleinenberg's Picro-sulphuric; glacial acetic with saturated aqueous picric acid and 1% chromic acid in water respectively in the proportions of 1, 15, and 5 parts by volume; saturated corrosive alcohol of 70%; Carnoy's fluid; and the fluid recommended by Looss with and without corrosive sublimate. The first two solutions had scarcely any penetrating power when cold but if warmed to steaming they killed rapidly but without allowing the worms to relax and also without very good preparation for histological examination. Saturated corrosive alcohol and Carnoy's fluid caused very bad collapsing but gave good penetration. For general purposes the best killing fluid was that of Looss--70% alcohol with 8% of its volume of glycerine--used steaming hot. It killed quite rapidly and had the virtue of allowing the worms to relax into a rather well extended state except the caudal region of the males which always curled up in a loose coil of two or three turns. Material intended for sectioning was either killed whole and cut into portions or first divided and the pieces killed and fixed which procedure avoided collapsing of the material during further treatment preparatory to sectioning.

(b) Preparation of Totos for Study

Specimens killed in glycerine and alcohol were allowed to remain in that fluid uncovered beneath a bell jar which permitted of a very slow concentration of glycerine by evaporation of the alcohol. When the fluid was quite thick, the specimens were removed to pure glycerine after which they were ready for study. Such specimens were sufficiently clear, except in the thicker regions of the body, for examination of internal organization. Greater transparency was ob-

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tained in other nematodes by clearing them in slightly warmed lacto-phenol. Any attempts at staining in toto and mounting in resinous media resulted, in most cases, in woeful and worthless preparations.

(c) Dissections and Teasing

Dissections of fresh nematodes were rendered very difficult by the tough cuticula and its refusal to tear except transversely. With the aid of a fine needle sharpened to an oblique chisel edge the cuticula could be rather easily divided along a mid-ventral, dorsal, or lateral line, in specimens which had stood for some time in lacto-phenol or glycerine. Dissection of fresh worms as mentioned above was difficult and of little value since the internal organs immediately disintegrated upon coming in contact with the water in which the operations were performed. Glacial acetic acid or a saturated solution of picric acid in water made good teasing fluids for fresh material--preferably the former, as it slightly hardened the tissues and at the same time improved their optical differentiation. In all dissection, the work was performed under a binocular microscope giving a good magnification.

(d) Sectioning

For sectioning the portions of the nematodes were very slowly run up thru the alcohol and cleared in xylol or chloroform, after which paraffin shavings were added bit by bit until the xylol was saturated. Then the pieces were transferred to a paraffin bath at 55° C. and imbedded from one-half to one hour according to their size. Sectioning and mounting on slides was then done in the usual manner. Difficulty was experienced in getting well preserved sections and considerable work must yet be done to that end.

(e) Staining

For staining purposes Ehrlich's hematoxylin and iron hematoxylin gave reasonable results although far from being perfect. Acid fuchsin and picric acid were used following iron hematoxylin to stain the plasma. With the above dyes difficulty was experienced when the sections were run into them and the lower alcohols by very often having the cuticular portion rise up or swell and tear away the other tissues; also there was poor nuclear differentiation. For rapid staining the sections were removed from 85% alcohol and the slide flooded with dibasic methylene blue in aqueous solution and allowed to stand until the tissues were deep blue. Then they were rinsed in water and flooded with a saturated watery solution of picric acid and left until the blue color was changed to a reddish purple. After this the sections were rapidly run up to xylol and destained in carbol-xylol, which turned the stain to a bright green, leaving the nuclei dark and a fair differentiation between tissues. After sufficient staining, the preparations were rinsed in xylol and mounted in damar.

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III External Characteristics of the Specimens

(a) Female

The female of Protospirura muris is a rather thick worm with a body which tapers anteriorly very rapidly to an extremely small head for the diameter and size of the nematode. Posteriorly the tapering is much less marked and the caudal portion terminates in a blunt wedge. They are white or slightly yellowish when fresh but have a tendency to turn brown after death. The cephalic and caudal ends are translucent viewed over a dark background and the oesophagus and smaller coils of the ovary show up very plainly as opalescent tubes. In freshly killed material, besides the coarse striations in the cuticula giving the worm an annulated appearance, there may sometimes be seen a very slight indentation running laterally along the worm, corresponding to the location of the lateral lines internally.

The length of female specimens seems to vary very widely as does also the width. Hall states they are 15--40 mm. long with a maximum diameter of 1.75 mm. Schneider makes 40 mm. as the maximum length; Marchi 44-46 mm.; while von Linstow gives 54 mm. for length and 1.9 mm. for the width. In the specimens examined for this paper the maximum length for the female was 52.5 mm. and the greatest width agreed with that of Hall, 1.75 mm.

The head of the nematode has two lips, tripartate corresponding in position to the dorsal and ventral surfaces of the animal. The nature of these lips and the three parts composing each will be explained later in connection with anatomical systems. In addition to the two lips there are on the head four papillae, circular in outline and placed one at the base of each external lobe of the tripartate lips a little laterally to the cleft separating the small lobes from

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the larger central one. These papillae have a diameter varying from 15.6 to 16.9μ for the larger specimens and a height of about 7.80 to 10.4μ. In the center of each papilla there is a rod-like structure which in sections takes a darker stain than the surrounding tissue and which seems to be homogeneous in nature. The papillae themselves are covered by a very thin cuticula and in some instances appear to be sunk into the surrounding tissue which rises around them as a shallow moat. In the female the excretory pore opens to the ventral surface of the animal in the mid-ventral line as a very obscure transverse slit. Its distance from the anterior end of the animal varies in individuals of different lengths--from 415μ in a 23 mm. specimen to 614μ in a 52.5 mm. worm.

The vulva has a similar variation in position according to the lengths of the individuals. In one case it was precisely in the center of the body between the anterior and posterior ends but in the other cases it was always just anterior to the mid-point of the body. As with the excretory pore, it opens in the ventral portion of the body in the mid-ventral line. Exteriorly the opening appears as a small transverse slit, in most cases difficult to find, in a slight elliptical depression in which the cuticula is more coarsely striated than in the surrounding parts.

The anus too opens ventrally by a long transverse slit at which point the maximum width of the body is 740μ nearly agreeing with Marchi's measurement of 0.7 mm. The anal opening does not precisely appear as a slit but more as if the cuticula had been invaginated or rolled inward at that point. The maximum distance of the opening from the caudal end is about 638μ, which is 108μ greater than the measurement given by Hall. The cuticula in this region very often ap-

pears as thickened or swollen, particularly on the dorsal side of the worm.

(b) Male

The male of this species is distinguished from the female by its smaller size both in length and diameter and also by the difference in the caudal end, but the general somatic appearance is the same. In preserved specimens this portion of the body is always coiled into a loose spiral of about two turns. The anterior end tapers similarly to that in the female but the posterior end suffers a slight swelling caused by the outgrowth of a right and left lateral ala. In the largest specimen, 32 mm. long, the left ala (right and left are reckoned from the dorsal aspect) began abruptly 24.5 mm. from the anterior end of the worm, while in the 28.5 mm. specimen it started at a distance of 19.5 mm. from the cephalic end. In the region of the anus the left ala is still distinctly wider than the right one. Here in different males there is considerable variation; in the largest ones the right alae measure 259--314.5μ in width and the left ones 166.5--203.5μ. The nature and characteristics of these will be described later under the general topic of cuticula. The maximum length of the males as given by Hall and Schneider is 28 mm. while the maximum found here is 32 mm. with a breadth of 740μ which is less than that given by either Hall or von Linstow. On the ventral caudal surface of the male there is a row of three papillae on each side so disposed that they converge toward each other and also toward the anal opening in a slowly tapering "V" (Fig. 11). Below the anus are two papillae on each side rather far apart and at the very posterior end are three pairs of small and almost inconspicuous papillae. Around the anal aperture is a rather heavy cuticular ridge which anteriorly

enlarges into an unpaired preänal papilla. It is however not as distinctive in appearance as the six paired ones. The terminations of the papillae are covered by a dome-shaped cap of thin cuticula and surrounded by a smooth ring of the same. Some of them scarcely rise above the surface of the surrounding alar cuticular while others project outward and in vertical section appear in outline like similar sections of the circumvallate papillae of the human tongue. The caudal end of the body proper of the male tapers rapidly to a point but its diameter is maintained larger than the maximum width of the body by the alae, which cause the tail to end rather bluntly. As the papillae are located out in the alar cuticula away from the body they are connected to the tapering body proper by stalks or evaginations of the hypoderm which can be easily seen thru the transparent involucre (Fig. 11).

The genital duct of the male has no separate opening to the exterior as does the vagina in the female but rather opens into that portion of the digestive tract slightly above the anus, the cloacal cavity.

The male too has the four cephalic papillae already described for the female. They are similar with the exception of size, which is about 11.36μ for diameter and 5.68μ for height. Also the excretory duct opens on the ventral surface in the mid-ventral line from 677μ from the anterior end in the largest specimen to 556.6μ in a 28 mm. individual--the latter measurement being quite closely in accord with the one given by Hall.

IV Anatomy

(a) Cuticula

The cuticula is the transparent, tough, colorless, striated covering enveloping the whole body of the nematode. It is continuous with the lips, the anal and oesophagal linings, although these latter show considerable variation in staining qualities. In fresh specimens the cuticula is sometimes thin and well applied to the body but at other times it appears swollen--raised in portions and wrinkled, particularly at the posterior end of both sexes. In preserved specimens the swollen portions have a tendency to become granular in appearance.

In the male the fine striations number about 299 per millimeter and 385 for the same unit in the female. These striations do not extend above the papillae on the head. On the alae of the male the fine striations leave off by an irregular line on the dorso-lateral portions of the tail where the alae fold outwards and are succeeded by very coarse and irregularly segmented striae which run obliquely from the sides of the body proper on the ventral surface forward to the edges of the alae, where they turn and go slightly caudad to the dorsal origin of the alae. Thus when the alae are viewed from either surface the impression given is that the surface is cut up into diamond-shaped areas or as if that portion of the body had been engine turned. These large striae are 11.3--16.9μ wide and in cross sections appear as rounded elevations 5.68--11.6μ high on a basement portion about 5.68μ thick with a distance of 2.6μ between each successive ridge.

The cuticula is made up of three main layers: An outer one, dense in nature; a middle stratum which appears sometimes as almost homogeneous, very finely granular, fibrous, densely granular;

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and an inner layer similar in nature to the outermost. Each of these has its own capacity for taking stain; the innermost stains more readily than the others and the outermost better than the middle layer except when the latter is coarsely granular at which time it stains very deeply. The inner layer is about 3.8--5.7μ thick and may be divided itself into three layers, the first and third clear and homogeneous taking stain less freely than the wide central portion. The same may be said for the external layer of cuticula, thus making seven layers in all for the body covering. The external lamina is only a trifle wider than the inner one so that most of the variations in thickness of cuticula are due to the middle layer since in some cases it may almost be lacking and in others be present to an extent of two or three times the sum of the thicknesses of the external and internal laminae.

The alae of the male nematode are formed by an outgrowth of the external layer and an enormous increase of the middle layer, which assumes a very coarse granularity.

In the spicular region each ala is supported on its dorso-lateral wall by a thickening of the cuticula which later pushes away from the external layer, approaches the body wall with the dorsally directed edge and the more ventral portion of the ala with the other edge. Thus in cross sections this structure appears as a rib-like process extending from the body across the alar space to its outermost margin (Fig. 8). This partition stains a trifle deeper than the surrounding portions of cuticula.

The cuticula is most variable in its action toward reagents; sometimes it will rise up forming large vesicles after the worms have been killed in glycerine and alcohol; in other cases where the speci-

mens have been so killed and later transferred thru evaporation to pure glycerine, the cuticula seems unaltered from its appearance in the living worm.

The cuticula is thicker generally over the mid-portion of the bodies and at the caudal ends--being thinnest near the cephalic region. In the body portion of the male the cuticula may vary from 38.8μ to 46.2μ, while in the head area the thickness is about 18.5μ. For the female the variation in the thickness at the central portion of the body is considerable, 37.0μ to 66.5μ, and in the anterior region 27.7μ to 46.5μ.

(b) Body-Cavity and Body-Wall

The body cavity of Protospirura muris is filled in the living state with a clear, rather thick fluid with the consistency of thin white of egg which is free to flow about during movements of the worm, as there are no mesenteric or other partitional supports holding the organs in place. In glycerine and lacto-phenol specimens this fluid is found in the cavity during dissections as a jelly-like substance very often gumming the organs together rendering their removal more difficult. The same substance appears in sections as granular matter or as a reticulum in the cavities between the different organs.

Just beneath the cuticula there is the hypoderm or subcuticula which forms a thin layer all around the body of the animal. At the mid-ventral and mid-dorsal regions as well as in the midlateral regions of the body, the hypoderm protrudes into the body cavity. In the first two locations, as seen in cross sections, it appears as a small bulge while in the lateral regions it projects far into the body as a two-lobed structure--often forming in places a sort

of support for the oesophagus and intestine but not quite in the manner Marchi suggests--that of completely clasping these tubes, altho they do approach such a condition. In the cephalic region, however, the four lines are equal in size and as he states, they are triangular with the apices directed toward the oesophagus and connecting with it forming four supports. The four bands remain nearly equal till a short ways from the nerve ring where the dorsal and ventral ones decrease in size. They continue with a slight variation in size--now larger, now smaller--till the caudal regions are approached, where in the male they appear very small but in the female they increase in size particularly the dorsal one. On the other hand, the lateral lines increase in size and then remain practically unaltered into the caudal regions. They appear to increase here and fill a greater part of the body cavity, but this is due rather to the decrease in size of the body itself at these places. In the female the lateral bands move slightly dorsad in the region of the anus, decrease in size and terminate at the tip of the tail; in the male they simply decrease and maintain their course to the end of the body.

The hypoderm is not divided into cells and contains no nuclei except where it bulges into the body cavity in the above-mentioned lines. Here it is freely nucleated with the majority of the nuclei lying in a group near the cuticular side of the band although they also appear scattered here and there in the projected portions. Nuclei in the other two lines do not appear very often but usually when they do so they are centrally placed.

Structurally the hypoderm appears merely as a granular layer of protoplasm. Where it forms the bands it is very coarsely granular, particularly in the lateral ones in which at the same time it

has a denser margin and a very thin clear covering appearing almost as a delicate cuticula. Furthermore the substance of the lateral bands is divided into two portions beginning below the nerve ring by a centrally placed partition which has a similar appearance to the limiting membrane, extending to the cuticula but appearing in no way to arise from it--which fact is in disagreement with the statement of Marchi, who believes it to be "but only a median prolongation of the involucre probably chitinous." In a thickening of the median partition there lies the excretory tube which will be described later.

(c) Head and Mouth-parts

As already mentioned in a previous part of this paper, the head of Protospirura muris terminates in two tripartate lips, one dorsal and one ventral (Fig. 1). When viewed en face the lips, although the head is round, give it the appearance of being almost rectangular in shape since the lobes of the lips line up as if to a straight edge leaving between the two lips a long narrow rectangular slit. With the surface of the lips slightly out of focus under the microscope their lobes arise as prism-like columns from beneath with their bases facing the exterior of the body. In cross sections of this region, the lobes are seen as heavily invested with cuticula continuous with the general body involucre and thru their centre is a cavity for the muscles used in moving the lobes to enlarge the oral aperture.

The two lateral divisions of each lip are from above roughly triangular in shape while the central lobe is square in outline. On the dorsal portion of the inner face of each lateral part (Fig. 3) there is a long, large and sharp cuticular tooth reflected backward, flanked on each side by a less large tooth directed obliquely back-

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ward, toward the margin of the lobe. These lateral teeth may be plain or notched and between them and the central tooth are one or more small serrations. Also there may be one or two very small serrations at its base laterally. The central lobes have a median tooth single pointed, di-notched, or serrated, flanked on either side by a variously notched tooth (Fig. 4). Sometimes the central tooth is not decidedly longer than the others and these latter may be so deeply notched as to make the armature appear as a row of five or more di-notched teeth. Usually, though, the center tooth is separated from the laterals by greater height and more prolonged downward marginal clefts. Also on the central lobes the cuticula is prolonged on each side into a sharp projection which appears as a lateral tooth.

In female worms the diameter of the head across the lip region varies with the size of the specimen from 185μ to 296μ with a more common measurement of 222μ. The same measurements for the male are 222μ to 259μ.

(d) Digestive System

The mouth, closed by the lips mentioned above, opens into a short pharynx very heavily lined with a deeply staining cuticula. In female worms the pharynx is from 142μ to 222μ long according to size of the individual, and for the male, 148μ to 180.5μ in large specimens. In cross sections it is round to vary slightly three-sided. From the pharynx there arises an oesophagus with a tripartate lumen extending thruout its length. Marchi states that there is a pharyngeal bulb formed by a swelling of the anterior part of the pharynx. In some specimens there is a very slight difference in size between the beginning of the oesophagus and the part following, while in others there is no difference at all. The variation is not strik-

ing enough to warrant calling the anterior portion a pharyngeal bulb and sections do not reveal any difference in structure between that and following parts. The oesophagus increases gradually but slightly to its insertion in the intestine by a three-lobed valve. In female specimens the thickness of the oesophagus in the mid-portion varied from 139.4μ to 323.μ and in the male, for large specimens, 240.5μ to 277.5μ. This portion of the digestive tube runs, including the valves, for a distance of 3.7 mm. to 5.1 mm. in the largest females and 2.59 mm. to 3.7 mm. in the males.

The outside covering of the oesophagus is a very thin homogeneous or amorphous tunic like a delicate cuticula. Internally its triradiate lumen is lined with a cuticula which takes stain very lightly. One radius of the lumen is pointed ventrally and the other two parts laterally so that the opening often appears as a "Y" when the portions are closely adjusted to each other.

Internally, as may be shown by cross sections, there are two kinds of muscle fibres in the oesophagus--those which Looss calls the "marginal" and "ordinary" fibres (Fig. 5). The former are more darkly staining fibres running in a bundle from each tip of the triradiate lumen to the amorphous membrane at the periphery. These fibres remain constant thruout the length of the organ while the ordinary fibres, on the other hand, arising from the sides of the triradiate lumen, are much more numerous and variable, also crossing to the outer tunica propria. Nuclei are scattered thruout, usually located nearer the periphery than the center of the mass in each third of the organ.

Between the ordinary fibres of the oesophagus are patches of granular protoplasm which become more regularly ordered in the posterior part of the oesophagus. They are undoubtedly the ramifications

of the oesophageal glands but without wax reconstruction their form can not be studied but must be left for further work. However, as a hint of their appearance, there showed in some cross sections three ducts--one centrally placed in each third of the oesophagus. About 18μ posterially, portions of horizontal canals connecting ducts appeared and then 36μ farther on there appeared six ducts, two in each portion, situated near each tip of the lumen on each side of it. Both in sections and in highly cleared toto preparations, three ducts opening into the oesophagus were seen; each one opening at a different level. Sections revealed that the first and anteriormost duct opened into the oesophagus from the dorsal segment, the second one from the right portion and the third from the left portion. Each duct opened by a short horizontal canal into one lined by the chitinous internal tunica of the oesophagus projected periferially from the mid-portion for a short distance (Fig. 5). In the female the three ducts opened respectively at distances of 900, 1737, and 2246μ from the anterior end, while in the male they entered at about 384, 493, and 642μ from the cephalic end. Marchi makes no mention of these openings at all in his description of the oesophagus nor of the glandular portions.

The oesophagus of the digestive tube opens into the intestine, called by Looss "the chyle intestine" and by Marchi the "stomaco-intestinale", by a valve. It has the appearance of having been thrust into the intestine so as to carry down a portion of it in such a manner that the latter is folded up very slightly upon the lower end of the oesophagus.

The valve is trilobed at its free end, but the divisions of these do not go down to the base of the valve altho the lumen is triradiate (Fig. 6). The lips are soft and covered by a delicate cuticula which in preserved specimens appears longitudinally folded.

The chyle intestine is covered by the same amorphous membrane as covers the oesophagus, and generally runs a straight course in both sexes to the region of the anus. However in young females and often in males too, the intestine is zig-zagged across the body particularly in the posterior region and in some instances even folded back on itself. There is very little variation in size of the intestine after its origin at the valve thru to the sphincter portion, being for the female about 370μ wide with a maximum of 462μ and 203.5μ for the male.

Viewed externally the intestine seems dappled as if made up of large irregularly placed cells. However, this appearance is due not to the size but to the variation of length in groups of cells lining the canal. Ordinarily the intestine occupies the center of the body cavity but is pushed to one side in the female when the uteri are crowded with eggs. In the lumen of the intestine are nearly always found finely divided granules which give a typical starch reaction with iodine and hence are undoubtedly particles of grain from the contents of the host's stomach.

The interior walls of the intestine appear as covered by randomly scattered mounds with little gullies running around and between them. In cross sections these mounds show up as constructed of very small columnar cells of graduated lengths giving the lumen of the canal a festooned aspect. This arrangement of cells is probably to increase the absorptive area of the chyle intestine much as the typhlosole in earthworms.

The nuclei of these columnar cells are nearly as broad as the cells themselves, oval and situated at the base of each near the tunica propria surrounding the tube. The cells when seen in cross

section are hexagonal like patches of honeycomb. The central ends of the cells are rounded and the whole interior surface of the tube is covered by a heavy, lightly staining layer, probably cuticular. In some specimens it appears as very finely striated at right angles to its surface while in others it is broken up into bristle-like fragments, causing a resemblance in some instances to ciliated epithelium. The cells of the intestine stain quite characteristically--dark at the base, lighter centrally and darker again at the tips, followed by a light area of the feebly staining lining.

The intestine proper ends at the sphincter muscle which surrounds and contracts it a little way anterior to the anal opening. In this region the columnar cells cease and the lumen becomes triangular and lined with cuticula.

The muscle tissue is thicker over the sides of the lumen, thinner at the corners and of course is circularly placed (Fig. 17). After the sphincter is passed the lumen becomes a long narrow cuticular-lined slit and continues as such till it opens to the exterior thru the anus. After the lumen has become a slit, a layer of hypoderm surrounds it and upon its dorsal surface muscle cells are laid down upon the sub-cuticula. As the anus is approached the muscle cells cease on the ventral portion of the body wall and the mid-ventral band melts into the hypoderm, which now completely surrounds the rectum (Fig. 13).

Apart from size the digestive tract is the same for the male except that the cloacal region is not so long and that the seminal duct opens into this portion just at the point on the dorsal surface where the sphincter leaves off and the chitinous cloaca begins. There are also other points of difference which will be described under the reproductive system.

(e) Excretory System

The excretory pore mentioned before opens in the mid-ventral line thru a small slit in the cuticula to the exterior of the nematode in both sexes. Its distance from the anterior end in the female is from 454μ to 614μ in large specimens and in the male 557μ to 688μ for big worms. Sections show that from the excretory pore the duct passes inward and downward for a short distance, when a process from the ventral half of each lateral band bearing a canal in its tip approaches the main canal where the three tubes fuse. The union duct is large with a much convoluted canal heavily lined with cuticula, which takes the stain as readily as the outer body covering (Fig. 7). After the union the canals lose their cuticular lining and continue down the lateral lines to the caudal ends of the animals where they disappear blindly. Histologically they are homogeneous, staining readily and exhibiting no peculiar nature. They are considered as single, long cells, with a central lumen, lying ordinarily in a thickening of the partition in the lateral bands at their innermost edges. However, they may, in different cases, move midway down the partition or off to one side in the substance of the bands themselves. The lumen is not constant; sometimes totally collapsed and very small, and at other times quite large; neither is the shape of the tubule constant, which may vary in sections from circular to a flattened oval shape.

At this time also, for convenience, may be mentioned in connection with lateral bands in a portion anterior to the excretory pore two large vesicular cells--one in each dorsal half of the bands. They begin far up in the lateral bands and extend just a little way below the junction of the two lateral line organs with the main excretory duct (Fig. 12). They are probably the cephalic glands so promi-

[illegible]

nent in some nematodes and if they have ducts, sections did not reveal them so their connection with the excretory duct--if they have such, as is the case in Agchylostoma duodenale--must be left for further observation.

(f) Nervous System

No attempt in this paper was made to study the nervous system except to note the position of the nerve ring and its appearance in sections. In the female this ring is from 382μ to 555μ from the cephalic end; for the male, 480μ to 518μ. In sections the nerve ring is made up of large vesicularly-nucleated cells staining deeply and appearing more or less in four groups separated by the lateral dorsal and mid-ventral lines which are equally prominent at this region. Also the ring is placed horizontally and not in a slanting position as in many other nematodes.

(g) Muscular System

Already in describing certain parts of this nematode certain muscles have been included in the discussion; namely, those in the pharynx, lobes of the lips and anal sphincters. Besides these may be mentioned those connected with the reproductive systems, which will be described in dealing with those organs, those of the external anterior pharyngeal region, the rectal muscles and those covering the body wall between the lateral and mid-ventral and dorsal lines.

In the most anterior cephalic region near the beginning of the pharynx, cross sections show that this portion of the tube is held in support by twelve muscle bundles--eight of which occur as four pairs, each couple corresponding in position to that occupied later by the mid-dorsal, mid-ventral and lateral lines (Fig. 2). The

other four appear as inter-radials between the four paired groups. Each muscle bundle extends from the tunica propria of the oesophagus, thru the circumoesophogeal tissue of this region, across the body cavity directly to the cuticula of the body wall. Very shortly the four unpaired bundles drop out but the grouped ones remain until the pharynx passes into the triradiate oesophagus where they seem to give way to the four supporting bands touching the oesophagus here. From this point on the tube is in connection with at least the lateral lines and by all four lines again in the region of the first appearance of the common excretory duct where it opens to the exterior. Apparently these external oesophageal muscles must be of assistance in some way during deglutition.

In the rectal region two very definite and strong muscles arise from the dorsal surface of the rectum, pass around the lateral bands and become inserted upon the body wall about midway between the dorsal and lateral lines (Fig. 13). Ventrally two muscles arise, but not so definitely, from the rectum and pass around in close connection with the enlarged mid-ventral line becoming as with the others inserted in the body wall, but this time on each side of the ventral line.

By far the most interesting musculature is that of the body proper by the use of which the animal performs its writhing worm-like movements. This is accomplished by muscle cells arranged longitudinally and side by side upon the hypoderm in the areas separating the four longitudinal bands--laterals, mid-ventral and dorsal lines.

The individual cells are very long fusiform ones tapering gradually to a fine point in the female but more abruptly in the male where the bodies are also about half as long. They are about 2.77 mm. long in females and 1.3 mm. in the male. In freshly teased specimens

of the nematode, it can be seen that the walls of the cells are thin but very tough and resistant to tearing and that the interior of the cell is filled with a finely granular and very liquid protoplasm which flows about the cell freely upon the slightest pressure deforming the cell wall. On the side next the hypoderm there is a darker strip of substance, the febrillar area, extending the length of the cell.

From these muscle cells on their dorsal surfaces arise lateral processes which traverse the body cavity to the dorsal or mid-ventral lines according as the cells are located in the dorsal or the ventral half of the worm (Fig. 16). Marchi makes the statement that a vesicular process arises from the center of each fusiform cell, passes over the contiguous cell and enters the next one. This is occasionally correct but generally more than one process arises from a cell--as many as five--passing always to the mid-lines with which they come in contact. These cross strands are non-fibrillar and filled by the same watery protoplasm occupying the greater part of the fusiform cell bodies. The muscle cells are also multi-nucleate, as many as 47 nuclei appearing in a single cell of a female individual.

In cross sections these fusiform cells appear resting in the hypoderm (Fig. 15) with the large vesicular portion protruding into the body cavity. The fibrillar layer appears as a heavily staining portion next the hypoderm often extending part way up the sides of the cell. Just above the fibrillar portion there is generally a condensation of granular protoplasm in which the nuclei rest. The remainder of the cell, that part projecting into the body cavity, looks like a large vacuole with a few granules of protoplasm scattered thru it. Sometimes however the whole interior of the cell may

be granular bearing a nucleus centrally placed. The vesicular processes never show nuclei and often very little protoplasm. Sections indicate that they do not appear to come into actual relationship with the mid-dorsal or ventral lines but rather only to come in contact with it, either at its tip or laterally. Varying numbers of sections of the cells appear in each quadrant but where they are largest, presumably at their centers, six cells occur to a quadrant. The tip ends of other cells may be seen squeezed in between the cells at their bases. At other times as many as twelve sections occur between bands but in such a case they are all small.

Marchi speaks of circular fibres also which seem to arise from the bodies of the fusiform cells. Such fibres do not appear and from other remarks he is undoubtedly calling the long vesicular processes circular muscles.

(h) Reproductive System

1 Male

The appearance of the caudal end of the male with its alae and papillae and the opening of the genital duct into the cloaca have previously been described so that there still remains for explanation the testes, the vas deferens and the spicular apparatus.

The testes is a single tube which extends anteriorally to within 6.9 mm. of the cephalic end. It begins as a blind rather sharply pointed tube a little way above the left ala which is 19 mm. to 24 mm. from the anterior limit just mentioned, turns and goes posteriad. Where the left ala arises the testes ceases and passes thru a brief constriction into the vas deferens which at the end of its course enters the cloaca.

The testis may be relatively straight thruout its course or slightly zig-zagged so that cross sections will pass thru the tube more than twice in some planes. The gonadal organs thruout their extent are covered by the same delicate amorphous tunica propria noticed as enveloping the digestive tract. Cross sections show that the walls of the testis in the anterior portion of the organ consist of small cells placed on the tunica while centrally there is a rachis around which the sperm cells are arranged. In the part just above the transition into the vas deferens the walls become thinner and the lumen is filled with spermatozoa appearing in sections as minute dots.

At the constriction between testis and vas deferens, the tunic becomes quite thick projecting in on each side about 9μ pushing in the lining cells and restricting the passage almost to a capillary one since the diameter of the whole tube at this point is 92.5μ. After this point is passed the tube becomes as wide as formerly--111μ and now is lined by rather narrow columnar cells pointing slightly downward or caudad. This portion of the tube is the vas deferens and retains the same structure till its opening into the cloaca.

Projecting into the cloacal cavity from the ventral wall, there is a slight prominence, cuticular in nature, bearing two openings very close together (Fig. 8). These are the spicular apertures thru which the spicules, during copulation, are protruded thence extending from the anus to the exterior of the body.

There are two spicules quite different in appearance. The right one is heavy walled and long--varying in males from 1.05 mm. to 1.11 mm. long by 27μ to 37μ wide. On the other hand the left spicule is very little narrower but measures in length a trifle less--from .629 mm. to .777 mm. Each spicule is encased in a transparent lamina

very prominent at its distal end making each one blunt, smooth and rounded. Each is also rather evenly curved and bears at its proximal end an enlargement for the attachment of muscles. Beneath the transparent lamina the dark walls of the spicule show up as relatively thick, and transversely striated, in particular for the right one. The left spicule reveals a central partition arising below the proximal expansion from its dorsal wall traversing the total length of the structure and becoming the dorsal wall itself 0.49 mm. from the distal end where the primary dorsal wall abruptly stops (Fig. 3). The spicules in sections show that they are not solid but instead filled with a pulp. Their distal ends beneath the thick lamella are covered with a tuft of sharp spines arising from the body wall of the spicule itself but these do not protrude thru the outer coating (Fig. 10).

Each spicule lies in a delicate sheath of cuticula continuous with the prominence mentioned as projecting into the cloaca, and is then totally enveloped in a thick muscular tunic--the exsertor muscle. It is attached distally to the cloacal prominence and proximally to the expanded portion of the spicule where it forms a slight bulb. Here also on each organ is attached the retractor muscle which passes obliquely to the dorsal wall of the body cavity upon which it is inserted, serving upon contraction to withdraw into its sheath the exserted spicule (Fig. 3).

(2) Female

In the female the reproductive organs consist of two ovaries two uteri, a vagina and vulva. The ovaries are two long, much-coiled tubes of slightly increasing calibre; one coils back and forth, sometimes encircling the intestine, in the anterior half of the nematode; the other, in the posterior half, often extends within a distance of

3 mm. from the caudal end.

The ovaries as shown by dissections end blindly as does the testis but not in a tapering point; they are merely rounded off abruptly. The average width of the ovarian tubes is about 37μ; however there is considerable variation thruout a single tube. Externally these tubes are covered by the same delicate tunica previously noted as enveloping the digestive tract and testis.

In cross sections the ovaries appear very similar to such sections of the testis in the male--with of course the difference that the elements are larger. There is a lining of low cuboidal cells around the walls of each tube and a central rachis upon which the germ cells are arranged. In longitudinal sections the germ elements are rather flat, piled closely one on top of the other and connected to the rachis.

The next portions of the female genital apparatus are the two uteri which are at their connection with the ovaries about three times the width of the latter. They, like the ovaries, are looped back and forth in their respective portions of the body according as they be connected with the anterior or posterior ovary. The uteri are often greatly distended with great numbers of eggs so that the body cavity is completely filled with the coils of these organs. In cross sections the cells lining the uteri are large ones showing two nuclei at their bases and a vesicular or more openly granular portion protruding into the lumen of the tube. Where the organs are crowded with eggs, these cells may be nearly obliterated giving the appearance in sections that the uteri are merely cuticular tubes loaded with embryo-carrying eggs. The eggs also appear to be embedded in a granular matrix which may possibly be only the coagulated fluid in the tubes simulating in killed material the granular deposits caused by the pre-

cipitation of the fluid filling the body cavity. The mature eggs contain embryos when deposited in a thick cuticular shell of the following dimensions: width, 29.4μ; length, 56.8μ; and thickness of shell 4--5μ.

The uteri may run parallel with each other for a short distance in an anterior-posterior direction before they become united with the vaginal region or they may diverge abruptly, one branch going anteriad and the other posteriad. As the uteri approach this point of junction the lining cells become more elongate, the tubes become narrower and the eggs are distributed along the canal singly. Also about 1.85 mm. from the junction the tubes become invested with a muscular coat of circularly placed muscle cells. These gradually become thicker as the uteri converge. Sometimes where the tubes seem to have joined and externally appear as a single tube, they retain their respective walls for a short distance and are not surrounded by a common muscle layer. At the junction the tube is about 185μ wide including the heavy circular muscular layer nearly 55.5μ thick in section. From the point where the uteri have a common opening the former type of elongate cells lining the canals stops and is replaced by a group of very long narrow cells, 369μ, with large swollen ends which project into the vagina proper. I am not certain of the exact number; there are at least seven. In the type species Protospirura numidica according to Seurat there are nine such cells which act as a valve permitting eggs to pass from the uteri but prohibiting any backward movement. Thruout this portion of the reproductive system there are in the circular muscle layers large granular areas circularly disposed which Seurat calls the sub-cuticular glands (Fig. 19).

The vagina proper is a sub-globular organ covered by the same tunica propria and muscle layer as enveloping the preceding

area while internally it is lined by a very thick spongy cuticula thrown up into large folds (Fig.18). This organ then opens to the exterior thru the vulva which has been described in another portion of this paper.

V Conclusions

1. Mature specimens show great variation in size which accounts for the conflicting measurements given by different authors.
2. The measurements given by Marchi and Hall for different regions and parts of Protospirura muris are included, with few exceptions, within the range of maximum and minimum measurements in this paper.
3. Protospirura muris agrees with the type species Protospirura numidica as described by Seurat, in the number and arrangement of the denticular processes on the lobes of the cephalic lips. The structuve of the vagina and neighboring parts of the uteri agree in general with the same portions in the type species.
4. Each of the three ducts of the oesophageal glands opens at a different level approximately 900μ apart in large female specimens and 150μ apart in males.
5. The spicules of the male are striated, unequal, and roughly in the proportion of right : left = 3 : 4. The right spicule is divided by a central partition for the greater part of its length.
6. The cuticula is composed of seven optically differentiated layers.
7. Sub-cuticular glands are present in the tissue of the utero-vaginal sphincter.

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VII Description of Plates

Abbreviations

al. r.	alar rib
alar sp.	alar space
bd. mus.	body muscles
cent. pt.	central partition
cent. t.	central tooth
ceph. gl.	cephalic gland
ceph. pap.	cephalic papilla
cloa. cav.	cloacal cavity
cm. ex. duct	common excretory duct
d. wl. end.	dorsal wall ending
ex. m.	exsertor muscle
gl. d.	glandular duct
inf.	infundibular swelling
int.	intestine
int. sph.	intestinal sphincter
inter. m. b.	inter-radial muscle bundle
l. l. org.	lateral line organ
lat. lb.	lateral lobe
lat. t.	lateral tooth
m. c.	muscular coat
oes. gl.	oesophageal gland
ov.	ovary
phar.	pharynx
pr. m. b.	paired muscle bundle
pr. an. p.	preanal papilla
rad. mus.	radial muscles

rect.	rectum
rect. m.	rectal muscle
retr. m.	retractor muscle
spic.	spicule
spn. sp.	spines of the spicula
sub. cut. gl.	subcuticular glands
sw. cut.	swollen cuticula
utr.	uterus
ut-vag. sph.	utero-vaginal sphincter
" " s.	" " "
vas def.	vas deferens
vag.	vagina

Plate I

Fig. 1 -- Top view of head. X 68

Fig. 2 -- Cross section of pharyngeal region showing extra-pharyngeal muscle bundles. X 615

Fig. 3 -- Lateral lobe of a lip. Exterior surface view. X 413

Fig. 4 -- Central lobe of a lip. Internal surface view. X 428

Fig. 5 -- Opening of duct of oesophageal gland into lumen of oesophagus. X 420

Fig. 6 -- Intestinal valve. Top view. X 68

Fig. 7 -- Anterior portion of common excretory duct. X 578

Fig. 8 -- Oblique section of caudal end of male showing part of spicular apparatus in the cloaca. X 128

Fig. 9 -- Outline drawing of left spicule. X 165

Fig.10 -- Tip of right spicule. X 25

Fig.11 -- Caudal end of male. X 60

Plate II

Fig. 12 -- Cross section showing lateral line organs and common excretory duct. X 180

Fig. 13 -- Cross section showing rectal muscles of the female nematode. X 120

Fig. 14 -- Diagram of utero-vaginal region. X 68

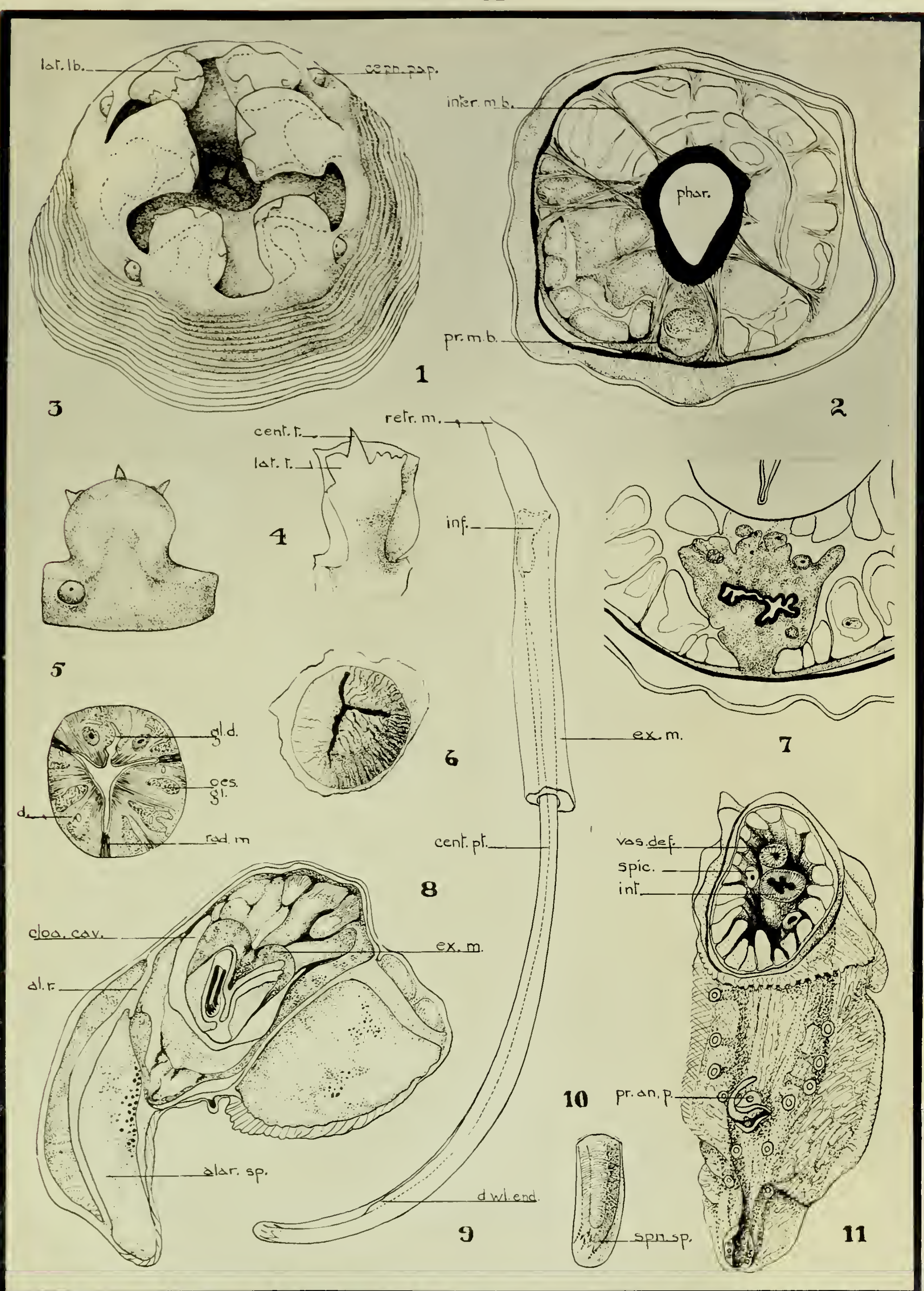
Fig. 15 -- Cross section of mid-portion of female. X 75

Fig. 16 -- Portion of a teased specimen showing vesicular processes of the body muscles approaching the mid-ventral line. X 90

Fig. 17 -- Cross section of female showing intestinal sphincter. X 83

Fig. 18 -- Cross section of vulvar opening of female. X 68

Plate I



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spn. sp.
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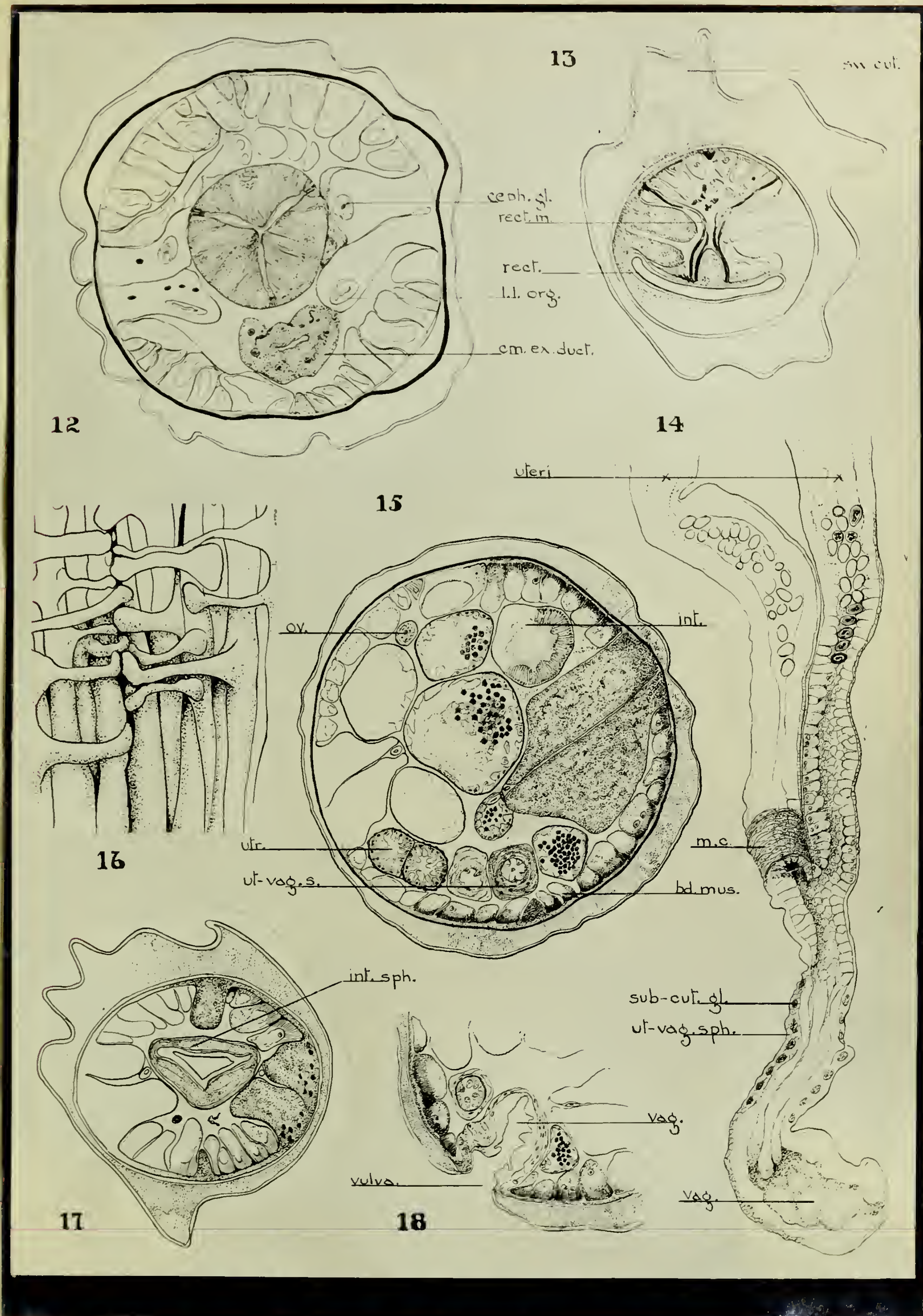
Plate II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